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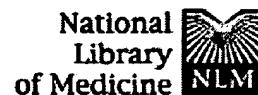
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## Human response to *Escherichia coli* O157:H7 infection: antibodies to secreted virulence factors.

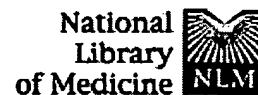
Li Y, Frey E, Mackenzie AM, Finlay BB.

Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3.

Vaccination has been proposed for the prevention of disease due to enterohemorrhagic *Escherichia coli* (EHEC), but the immune response following human infection, including the choice of potential antigens, has not been well characterized. To study this, sera were obtained from five pediatric patients with acute diarrhea caused by *E. coli* O157:H7 0, 8, and 60 days after hospitalization. These sera were used to examine the immune response to four different EHEC virulence factors: Tir (translocated intimin receptor, which is inserted into the host cell membrane), intimin (bacterial outer membrane protein which binds to Tir), EspA (secreted protein which forms filamentous structures on EHEC surface), and EspB (inserted into the host membrane and cytoplasm). The response to O157:H7 lipopolysaccharide was also examined. Sera were assayed against purified recombinant proteins using immunoblot analysis and by enzyme-linked immunosorbent assay to determine the sera's titers to each of the antigens in all patients. We found that there was little reaction to EspA, EspB, and intimin in the acute-phase sera, although there was some reactivity to Tir. By day 8, titers of antibody to all four virulence factors were present in all patients, with a very strong response against Tir (up to a titer of 1:256,000), especially in hemolytic-uremic syndrome patients, and lesser strong responses to the other three antigens. The titer to the antigens 60 days after hospitalization was decreased but was still highest for Tir. These results suggest that there is a strong immune response to Tir, and to a lesser extent to the other three virulence factors, following EHEC disease, indicating that these bacterial molecules are potential vaccine candidates for preventing EHEC disease. They also suggest that bacterial virulence factors that are inserted into host cells during infection by type III secretion systems (Tir or EspB) are still recognized by the host immune response.

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## Prevention and control of enterohaemorrhagic *Escherichia coli* (EHEC) infections: memorandum from a WHO meeting. WHO Consultation on Prevention and Control of Enterohaemorrhagic *Escherichia coli* (EHEC) Infections.

Reilly A.

World Health Organization, Geneva, Switzerland.

*Escherichia coli* is a commonly occurring inhabitant of the intestine of humans and other animals, but there are several pathogenic types of *E. coli* which cause a variety of human diseases. One of these pathogenic types, *E. coli* O157:H7, belongs to the group of enterohaemorrhagic *E. coli* (EHEC) which produce potent toxins and cause a particularly severe form of disease, haemorrhagic colitis (HC). About 10% of patients with HC can go on to develop haemolytic uraemic syndrome (HUS), a life-threatening complication of *E. coli* O157:H7 infection that is characterized by acute renal failure, haemolytic anaemia, and thrombocytopenia. These sequelae are particularly serious in young children and older people. On average, 2-7% of patients with HUS die, but in some outbreaks among the elderly the mortality rate has been as high as 50%. This Memorandum reviews the growing importance of *E. coli* O157:H7 as a foodborne pathogen and reports on the issues of surveillance, outbreak investigation, and control strategies with respect to EHEC infections that were discussed at the WHO Consultation on Prevention and Control of EHEC Infections, held in Geneva on 28 April to 1 May 1997. Recommended measures for prevention and control include the following: use of potable water in food production; presentation of clean animals at slaughter; improved hygiene throughout the slaughter process; appropriate use of food processing measures; thorough cooking of foods; and the education of food handlers, abattoir workers, and farm workers on the principles and application of food hygiene.

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## Immunization-

Haneefah Muhammad's presentation of:

Potter, Andrew A., Klashinsky, Sandra., Li, Yuling., Frey, Elizabeth., Townsend, Hugh., Rogan, Dragon., Erickson, Galen., Hinkley, Susanne., Klopfenstein, Terry., Moxely, Rodney A., Smith, David R., Finlay Brett B. 2004. Decreased shedding of Escherichia coli 0157:H7 by cattle following vaccination with type III secreted protein. *Vaccine*. 22, 362-369.

Fig: Paper Citation

## Big Question

Is it possible to vaccinate cattle to decrease the level of E-coli 0157:H7 shedding for the purpose of reducing the risk of human disease?

## Study's Objective

To determine if vaccination of cattle can be used as an adequate strategy in reducing the number of this strain of E-coli bacteria shed from cattle.

Cattle and their products are associated with the majority of cases of E-coli 0157:H7 infection in humans.

Causes severe diarrhea and in a small number of cases haemolyticuremic syndrome (HUS)

The number of animals shedding the organism in their feces is usually higher in the summer months which correlate with an increased incidence of human disease.

## What is Immunization?

Immunization is when an individual is exposed to an antigen which induces their body to produce antibodies.

## Vaccination and Experimental Infection

Calves and yearling (adult) cattle were obtained

All animals were screened for serum antibody titers against E-coli secretory proteins and shedding of the E-coli.

Trial 1: Eight calves were vaccinated twice on different days while another 8 calves were given a placebo on the same days.

Trial 2: Three groups of yearling cattle were immunized on the same days with a supernatant, a Tir mutant, or a placebo.

Two weeks following the last vaccination all cows were infected with E-coli and fecal shedding of the organism was monitored for fourteen days.

## Protection from Natural Exposure

Trial 3: One hundred ninety two steers blocked by weight were placed in four pens (3 weight groups per pen). Two pens were vaccinated while two weren't vaccinated.

Fecal matter was collected for bacterial culture.

Outcome was measured as pen-level performance.

## Results

Trial 1: The eight calves that were vaccinated showed a 13 fold increase in specific antibody titre

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compared to the placebo group after a single immunization. After a booster vaccination it showed a 45 fold increase. While only one placebo calf seroconverted.

Seven of eight placebo-immunized animals shed the bacteria during the trial and 4 of those animals shed for four or more consecutive days. On the other hand 5 of 8 shed bacteria during the trial and only 1 shed the bacterium for more than two consecutive days.

The total number of bacteria isolated from fecal samples was significantly lower among the vaccinated group compared to the placebo group, the former having 6.25 colony forming units (CFU) per gram of feces and 81.25CFU/g for the latter.

Fig. 1 Number of calves shedding E-coli on each experiment day

Trial 2: The group that received the mutant Tir showed a response similar to the group that got the vaccine from the wild-type. Though on one particular day there was an increase in anti-Tir antibody titre in the mutant Tir group as well as the placebo group. It was observed that there was an organism producing an immunologically-related molecule or natural exposure to E-coli. Also, the placebo group shed the organism for a median of 4 days compared to zero days for the other two groups.

Trial 3: The pre-treatment prevalence of shedding was 30%. After treatment the placebo pens shedding was 21.3%, and the vaccine pens were significantly lower at 8.8% .

Fig. 2 The proportion of cattle shedding the E-coli within pens of vaccinated and unvaccinated cattle in conditions of natural exposure.

### What it Means

Since it was shown that when the number of E-coli 0157:H7 increased in the environment incidence of disease also rose. This study has demonstrated that the vaccination of cattle can be used to reduce the number of E-coli 0157:H7 shed from cattle as a way of preventing disease.